ZooKeys 1198: 185–192 (2024)
DOI: 10.3897/zookeys.1198.112618

Research Article

# A new chemosymbiotic bivalve species of the genus *Acharax* Dall, 1908 (Bivalvia, Solemyida, Solemyidae) from the Haima cold seep of the South China Sea

Mei Yang[1*], Baoquan Li[2*], Zhibin Gan[1], Dong Dong[1], Xinzheng Li[1,3,4]

1 *Department of Marine Organism Taxonomy & Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*
2 *Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China*
3 *Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China*
4 *University of Chinese Academy of Sciences, Beijing 100049, China*

*Corresponding authors: Baoquan Li (bqli@yic.ac.cn); Xinzheng Li (lixzh@qio.ac.cn)*

## Abstract

Solemyidae is an ancient group of protobranch bivalves that typically inhabit unusual environments, such as deep-sea chemosynthetic environments, and are symbiotic with chemoautotrophic and gill-hosted bacteria. In May 2018, a living solemyid bivalve was collected using a remotely operated vehicle at a depth of 1,390 m from the Haima cold seep in the northwestern slope of the South China Sea. Through a comprehensive taxonomic approach combining morphological observations and molecular phylogeny reconstruction of concatenated mitochondrial COI,16S rRNA and 18S rRNA gene sequences, a new species, *Acharax haimaensis* **sp. nov.** is identified and described. The discovery of this new species contributes to the diversity of known solemyids in deep-sea chemosynthetic environments.

**Key words:** *Acharax haimaensis* sp. nov., Bathyal, deep-sea, taxonomy

OPEN ACCESS

Academic editor: Fedor Konstantinov
Received: 13 September 2023
Accepted: 22 March 2024
Published: 24 April 2024

ZooBank: https://zoobank.org/867D3E4F-FCC1-4BFD-BEA2-AC7704C01E1D

Citation: Yang M, Li B, Gan Z, Dong D, Li X (2024) A new chemosymbiotic bivalve species of the genus *Acharax* Dall, 1908 (Bivalvia, Solemyida, Solemyidae) from the Haima cold seep of the South China Sea. ZooKeys 1198: 185–192. https://doi.org/10.3897/zookeys.1198.112618

Copyright: © Mei Yang et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

## Introduction

Solemyidae is a group of ancestral protobranch bivalves (Pojeta 1988) with a worldwide marine distribution across various depths ranging from 0 to 6,000 m (Conway et al. 1992; Fujiwara et al. 2003). This family consists of over 30 valid living species that are classified into two reciprocally monophyletic genera: *Acharax* Dall, 1908 and *Solemya s.l.* Gray 1840. These genera are distinguished by their unique thickened frill of radially pleated periostracum, which extends beyond the calcified shell margins. The main difference between these genera lies in the position of the ligament; it is external in *Acharax* and internal in *Solemya s.l.* (Taylor et al. 2008; Kamenev 2009; Oliver et al. 2011; Sharma et al. 2013). Solemyid bivalves predominantly inhabit chemosynthesis-based ecosystems (Conway et al. 1992; Walton 2015; Bailey 2021) and rely on intracellular chemosynthetic symbionts for nutrition (Fisher and Childress 1986; Rodrigues et al. 2010; Fukasawa et al. 2017).

* These authors contributed equally to this work.

The classification of Solemyidae based on external features is problematic (Kamenev 2009; Oliver et al. 2011; Bailey 2021) due to the lack of distinguishing characteristics such as shell sculpture and hinge teeth. These bivalves are uniformly covered by a distinctive, thick, shiny periostracum (Taylor et al. 2008). Furthermore, there has been limited research on the morphology and molecular aspects of solemyid bivalves. As a result, the taxonomy and systematic status of Solemyidae have remained problematic. Recently, Sato et al. (2013) described and depicted the shell microstructures of five solemyid species from Japan by scanning electron microscopy.

In this study, we diagnose and describe a new *Acharax* species from the Haima cold seep, China. Additionally, we conducted an analysis of interspecific genetic distances within the family Solemyidae based on the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Furthermore, we examined the phylogenetic relationships within the order Solemyida using COI,16S rRNA and 18S rRNAgene sequences.

## Materials and methods

The specimen was collected from the Haima cold seep in the northwestern slope of the South China Sea at a depth of 1,390 m using a remotely operated vehicle (ROV) in 2018 (Fig. 1). On board, the specimen was photographed with a Canon EOS-1D digital single lens reflex camera. Then, the specimen was fixed in 95% ethanol and deposited in the Marine Biological Museum of the Chinese Academy of Sciences (**MBMCAS**) in the Institute of Oceanology, Chinese Academy of Sciences, Qingdao. Measurements were taken point-to-point with digital calipers, recorded to the nearest 0.1 mm.

Total genomic DNA was extracted from the muscle tissues using TIANamp Marine Animals DNA Kit (TIANGEN, China) following the manufacturer's instructions. Then the genomic DNA was used for Illumina sequencing and Oxford Nanopore sequencing (Shanghai BIOZERON Co. Ltd). After assembly and annotation, we successfully obtained the complete mitochondrial genome of the new *Acharax* specimen (GenBank accession number: ON023263).

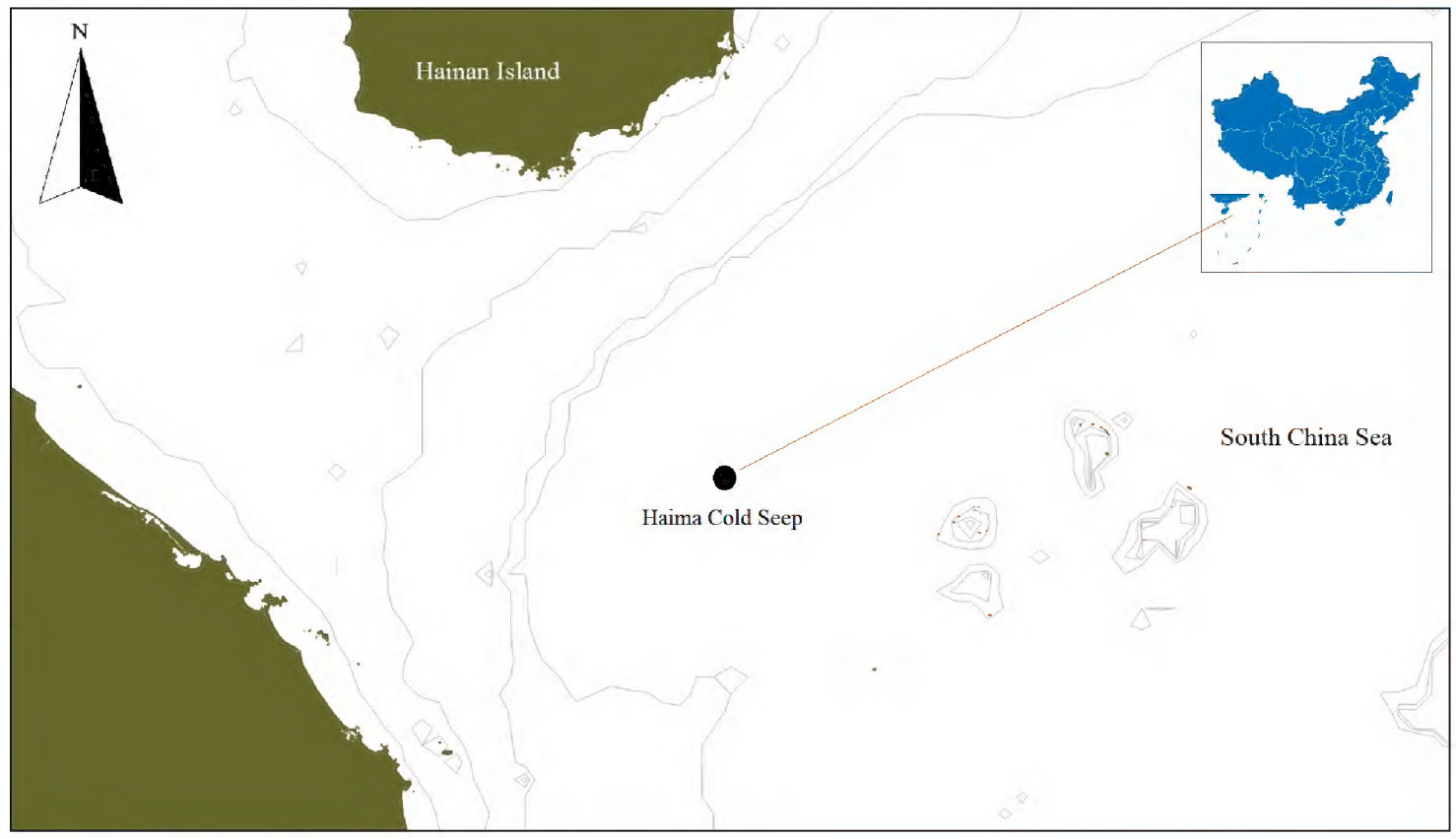


**Figure 1.** Sampling site of *Acharax haimaensis* sp. nov.

The Kimura's 2-parameter (K2P) genetic distances between COI sequences of solemyid species were estimated by MEGA 6.06 (Tamura et al. 2013). The phylogenetic relationships within Solemyida were conducted using COI, 16S rRNA and 18S rRNA gene sequences from 17 in-group species (Suppl. material 1). The nucleotide sequences of each gene were aligned in batches using MAFFT (Katoh et al. 2019), and ambiguously aligned regions were deleted using Gblocks 0.91b (Gblocks parameters: minimum length of a block = 5; allowed gap positions = with half) (Talavera and Castresana 2007). Subsequently, the sequences were concatenated into a single alignment used to generate nexus files in PhyloSuite 1.2.2 (Zhang et al. 2020). A maximum-likelihood analysis was performed with the GTR+I+G substitution model in IQ-TREE (Nguyen et al. 2015), and branch support was evaluated with ultrafast bootstrap (UFB) with 1,000 replicates. The phylogenetic tree and node labels were graphically edited with iTOL (Letunic and Bork 2007).

## Results

### Systematics

**Order Solemyoida Dall, 1889**
**Superfamily Solemyoidea Gray, 1840**
**Family Solemyidae Gray, 1840**

**Genus *Acharax* Dall, 1908**

**Type species.** *Solemya johnsoni* Dall, 1891; Recent, North Pacific.

***Acharax haimaensis* sp. nov.**
https://zoobank.org/2356CE0D-13A0-4AA7-A322-D5ACD518F028
Fig. 2

**Material examined. *Holotype*:** Complete, Haima cold seep cruise, HOV *Shen Hai Yong Shi* 71, 1390 m, coll. crew of R/V *Tan Suo Yi Hao*, 17 May 2018, MBM287872.

**Description. *Shell*:** the shell is elongate, rectangular, equivalve, and robust. Shell length 103.32 mm, width 48.68 mm, height 45.20 mm. The length/width ratio and length/height ratio are 2.12 and 2.29, respectively. The beak is positioned at approximately the posterior quarter of the shell. The anterior margin is broadly rounded and compressed medially, while the posterior margin is tightly rounded. The ventral margin is straight and shallowly concave towards the posterior. The periostracum extends well beyond the calcified shell margin, folding inwards, and reddish brown but gradually paler towards the prodissoconch. The shell has radial ridges with strong, flattened summits. The ridges are more crowded anteriorly and posteriorly, and vary in both width and colour. There are four closely spaced ridges over the posterior area, and the median area is almost smooth, with two or three low ribs. The anterior region with eight or nine deeply cut ribs. Hinge teeth are absent, and the ligament is mainly external. The posterior siphon aperture is lined by two rings of papillae. The foot is

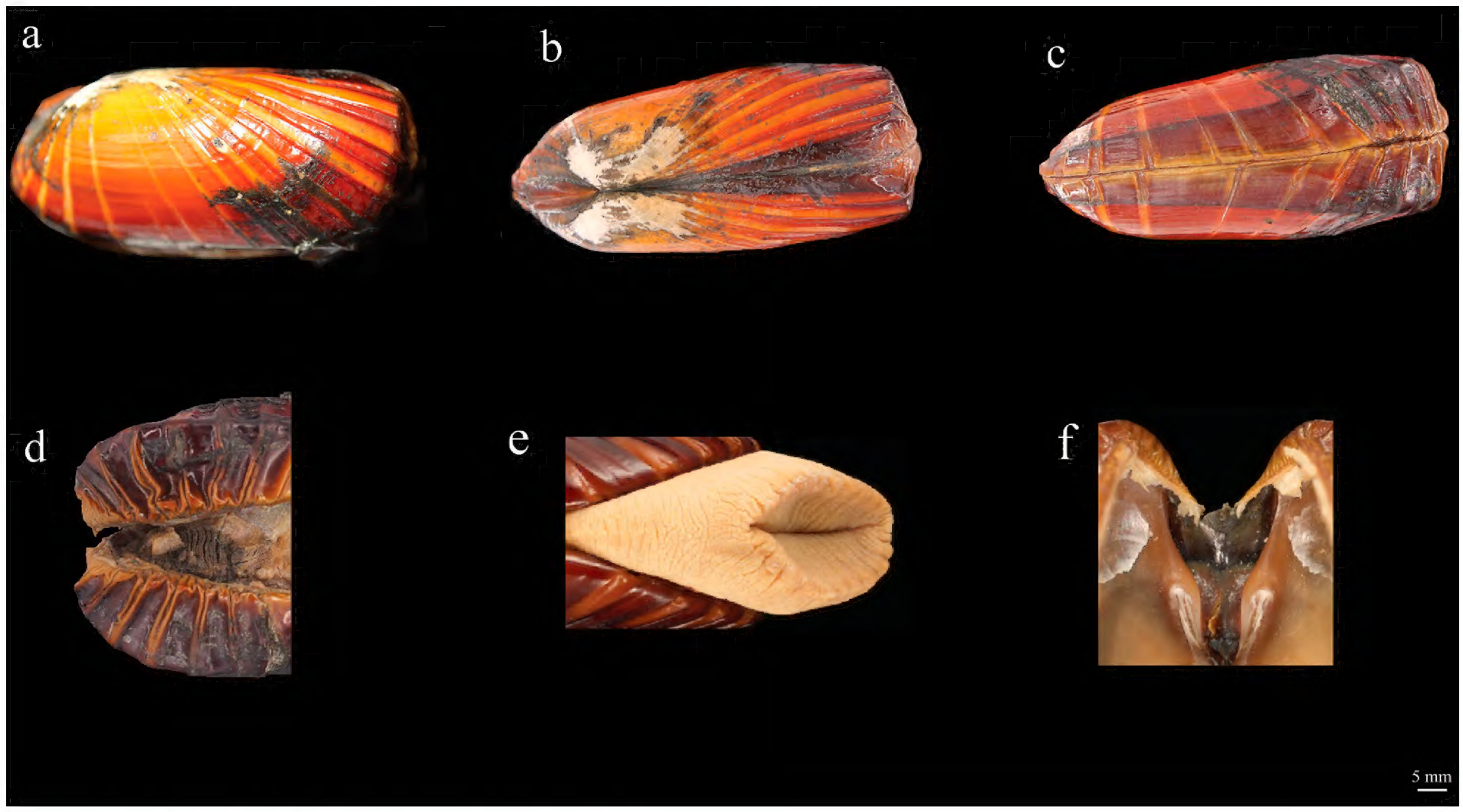


**Figure 2.** Holotype of *Acharax haimaensis* sp. nov.

large, with a broadly oval sole, and its margin interdigitates between large and small blunt papillae.

***Adductor muscle scar and pallial scars*:** the anterior adductor-muscle scar has a D-shaped, irregularly and posteriorly oriented straight face. There is a loop of muscle scar that extends from the hinge plane, reaching halfway along the valve and connecting ventrally to adductor scar. The posterior adductor scar is ovoid, with irregular contours, and exhibits faint radial sculpture. It is separated by a line that extends from the scar to the beak along a subtle depression. The foot is flattened at anterior end when open. The flattened face is longitudinally bifurcated, displaying horizontal lamellae, and is fringed with approximately 32 short, adze-shaped ridges. These ridges interlock when the foot is closed, and each ridge is topped with a small, rounded papilla. The mantle is fused along the ventral margin and covers large gills that extend about three-fifths of the total length of the body.

**Etymology.** Named after the type locality, the Haima cold seep in the northwestern slope of the South China Sea.

**Distribution.** Currently, *Acharax haimaensis* sp. nov. is known only from the type locality, in the northwestern slope of the South China Sea, at a depth of 1,390 m.

**Remarks.** The genus *Acharax* has a worldwide distribution in cold seep habitats with sulfide present (Sibuet and Olu 1998) at depths ranging from approximately 400 m to 6,000 m (Neulinger et al. 2006). *Acharax* has approximately nine extant species (WoRMs 2024) and more than 20 fossil seep species (Amano and Ando 2011; Saether et al. 2016; Isaji and Kato 2017; Hansen et al. 2020). *Acharax clarificata* Dell, 1995 closely resembles to our specimen, but the new species differs from *A. clarificata* in the shape of the shell. Our specimen has an elongate, nearly rectangular shell with parallel dorsal and ventral margins, whereas *A. clarificata* has a shallowly concave ventral margin and is particularly more deeply concave towards the posterior end.

The genus *Acharax* exhibits distinct morphological differences from *Solemya*, including a prominent large external ligament located on a narrow nymph and larger size (Walton 2015). However, due to the striking similarities in gross morphology with *Solemya*, there is a possibility that *Acharax* species have been misclassified as *Solemya* in the past (Sibuet and Olu 1998). Even within the genus *Acharax*, certain species share morphological similarities, but molecular data suggests the presence of cryptic speciation (Neulinger et al. 2006). Therefore, gene-sequence analysis can provide valuable information for the classification of solemyid bivalves.

**Molecular support.** The genetic divergence between *Acharax haimaensis* sp. nov. and the solemyid species analyzed ranged from 21.4% (*A. johnsoni*) to 28.2% (*Solemya pusilla*) (Table 1). It is evident that the lowest genetic distance was observed between the two *Acharax* species. The phylogenetic tree of the family Solemyidae, reconstructed using maximum likelihood based on mitochondrial COI,16S rRNA and 18S rRNA sequence data, is shown in Fig. 3. Both *Acharax* and *Solemya* formed monophyletic clades with strong support values (≥95%).

The placement of the new species, *Acharax haimaensis* sp. nov., within the genus *Acharax* is supported by both the morphological and molecular phylogenetic analyses. Its discovery at the Haima cold seep contributes to the known diversity of solemyids in chemosynthesis-based ecosystems. The Protobranchia represent an intriguing group of Bivalvia in terms of their early evolution, unique anatomy, larval development, and ecological diversification. However, there are still controversies surrounding the origin and evolutionary process of Protobranchia, particularly the phylogenetic relationships among higher taxa. Currently, research into the morphological taxonomy and molecular systematics of the Protobranchia is relatively limited. More comprehensive taxon collections in the future will be necessary to lead us closer to the goal of reconstructing the evolutionary history of protobranch bivalves.

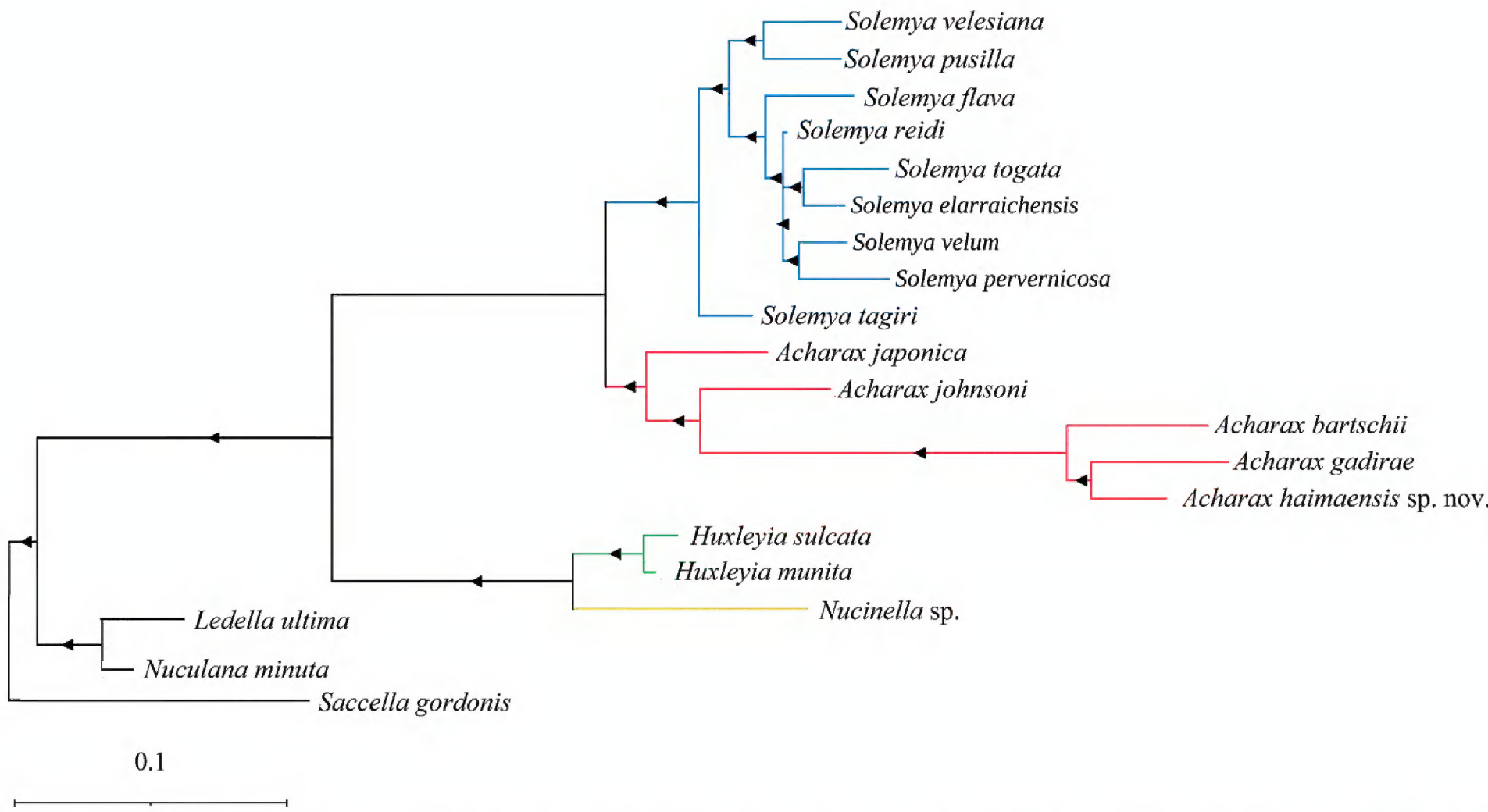


**Figure 3.** Phylogenetic relationships of Solemyida by the ML analysis of mitochondrial (COI+16S rRNA+18S rRNA) sequences. The black triangles demonstrate bootstrap values ≥95% for the node.

**Table 1.** Kimura's 2-parameter pair-wise genetic distances (in percentage) between species of Solemyidae using COI gene sequences.

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| *Acharax haimaensis* | – | | | | | | | | |
| *Acharax johnsoni* | 21.4 | – | | | | | | | |
| *Solemya elarraichensis* | 27.8 | 28.0 | – | | | | | | |
| *Solemya flava* | 25.8 | 29.5 | 18.4 | – | | | | | |
| *Solemya pervernicosa* | 23.4 | 22.6 | 26.4 | 24.5 | – | | | | |
| *Solemya pusilla* | 28.2 | 25.0 | 17.3 | 20.4 | 27.7 | – | | | |
| *Solemya tagiri* | 25.1 | 22.7 | 19.8 | 19.6 | 25.1 | 18.8 | – | | |
| *Solemya velesiana* | 25.6 | 24.0 | 17.7 | 19.6 | 24.6 | 12.2 | 15.2 | – | |
| *Solemya velum* | 27.5 | 29.6 | 16.4 | 17.8 | 29.1 | 18.7 | 16.5 | 15.6 | – |

## Acknowledgements

The authors thank the captain and crew of the R/V *Tan Suo Yi Hao* and the pilots of the HOV *Shen Hai Yong Shi* for providing technical support. This work is financially supported by the Senior User Project of R/V Kexue (No. KEXUE2020 GZ01) and the National Natural Science Foundation of China (No. 42176114).

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by Institute of Oceanology, Chinese Academy of Sciences and National Natural Science Foundation of China

### Author contributions

Mei Yang, Baoquan Li, Zhibin Gan, Dong Dong and Xinzheng Li designed the study. Xinzheng Li collected the specimen. Mei Yang, Baoquan Li and Xinzheng Li examined the specimens, performed morphological comparisons, and conducted the molecular analysis. Zhibin Gan and Dong Dong was involved in contributions to the conception of the work and took the photos of the specimen. All authors prepared the manuscript, read, and approved the final version.

### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

## References

Amano K, Ando H (2011) Giant fossil *Acharax* (Bivalvia: Solemyidae) from the Miocene of Japan. The Nautilus 125: 207–212.

Bailey JB (2021) The genera that never were: the impact of *Janeia* and *Janecikia* on phyletic and taxonomic relations within the Solemyidae (Bivalvia: Protobranchia). Palaeontologia Electronica 24: a12. https://doi.org/10.26879/945

Conway NM, Hows BL, McDowell Capuzzo JE, Turner RD, Cavanaugh CM (1992) Characterization and site description of *Solemya borealis* (Bivalvia; Solemyidae), another bivalve-bacteria symbiosis. Marine Biology 112(4): 601–613. https://doi.org/10.1007/BF00346178

Fisher CR, Childress JJ (1986) Translocation of fixed carbon from symbiotic bacteria to host tissues in the gutless bivalve *Solemya reidi*. Marine Biology 93(1): 59–68. https://doi.org/10.1007/BF00428655

Fujiwara Y (2003) Symbiotic adaptation for deeper habitats in chemosynthetic environments. The Journal of Geography 112(2): 302–308. https://doi.org/10.5026/jgeography.112.2_302

Fukasawa Y, Matsumoto H, Beppu S, Fukasawa Y, Kawato M, Miyazaki JI (2017) Molecular phylogenetic analysis of chemosymbiotic Solemyidae and Thyasiridae. Open Journal of Marine Science 7(1): 124–141. https://doi.org/10.4236/ojms.2017.71010

Hansen J, Ezat MM, Åström EKL, Rasmussen TL (2020) New late Pleistocene species of *Acharax* from Arctic methane seeps off Svalbard. Journal of Systematic Palaeontology 18(2): 197–212. https://doi.org/10.1080/14772019.2019.1594420

Isaji S, Kato H (2017) A chemosymbiotic molluscan assemblage from the Miocene Amtsu Formation in the University of Tokyo Chiba Forest, Boso Peninsula, central Japan. Journal of the Natural History Museum and Institute, Chiba 10(Special Issue): 45–50. https://gbank.gsj.jp/ld/resource/geolis/201750903

Kamenev GM (2009) North Pacific species of the genus *Solemya* Lamarck, 1818 (Bivalvia: Solemyidae), with notes on *Acharax johnsoni* (Dall, 1891). Malacologia 51(2): 233–261. https://doi.org/10.4002/040.051.0202

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Letunic I, Bork P (2007) Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics, 23(1): 127–128. https://doi.org/10.1093/bioinformatics/btl529

Neulinger SC, Sahling H, Sühling J, Imhoff JF (2006) Presence of two phylogenetically distinct groups in the deep-sea mussel *Acharax* (Mollusca: Bivalvia: Solemyidae). Marine Ecology Progress Series 312: 161–168. https://doi.org/10.3354/meps312161

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Oliver G, Rodrigues CF, Cunha MR (2011) Chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz, NE Atlantic, with descriptions of new species of Solemyidae, Lucinidae and Vesicomyidae. ZooKeys 113: 1–38. https://doi.org/10.3897/zookeys.113.1402

Pojeta JJ (1988) The origin and Paleozoic diversification of solemyoid pelecypods. New Mexico Bureau of Mines and Mineral Resources, Memoir 44: 201–271.

Rodrigues CF, Webster G, Cunha MR, Duperron S, Weightman AJ (2010) Chemosynthetic bacteria found in bivalve species from mud volcanoes of the Gulf of Cadiz. FEMS Microbiology Ecology 73: 486–499. https://doi.org/10.1111/j.1574-6941.2010.00913.x

Saether KP, Sha J, Little CTS, Campbell KA (2016) New records and a new species of bivalve (Mollusca: Bivalvia) from Miocene hydrocarbon seep deposits, North Island, New Zealand. Zootaxa 4154(1): 1–26. https://doi.org/10.11646/zootaxa.4154.1.1

Sato K, Nakashima R, Majima R, Watanabe H, Sasaki T (2013) Shell microstructures of five Recent solemyids from Japan (Mollusca: Bivalvia). Paleontological Research 17(1): 69–90. https://doi.org/10.2517/1342-8144-17.1.69

Sharma PP, Zardus JD, Boyle EE, Gonzalez VL, Jennings RM, McIntyre E, Wheeler WC, Etter RJ, Giribet G (2013) Into the deep: A phylogenetic approach to the bivalve subclass Protobranchia. Molecular Phylogenetics and Evolution 69(1): 188–204. https://doi.org/10.1016/j.ympev.2013.05.018

Sibuet M, Olu K (1998) Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-sea Research. Part II, Topical Studies in Oceanography 45(1–3): 517–567. https://doi.org/10.1016/S0967-0645(97)00074-X

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Taylor JD, Glover EA, Williams ST (2008) Ancient chemosynthetic bivalves: Systematics of Solemyidae from eastern and southern Australia (Mollusca: Bivalvia). Memoirs of the Queensland Museum 54: 75–104.

Walton K (2015) New Zealand living Solemyidae (Bivalvia: Protobranchia). Molluscan Research 35(4): 246–261. https://doi.org/10.1080/13235818.2015.1053168

WoRMs (2024) World Register of Marine Species. *Acharax* Dall, 1908. https://www.marinespecies.org/aphia.php?p=taxdetails&id=293237 [Accessed on: 2024-03-27]

Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

## Supplementary material 1

### The mitochondrial gene sequences used in this study

Authors: Mei Yang, Baoquan Li, Zhibin Gan, Dong Dong, Xinzheng Li

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1198.112618.suppl1